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THE EFFECT OF ANAPHYLACTIC SHOCK ON THE CELLULAR REACTION OF THE PERITONEUM OF THE GUINEA-PIG *

FRANCIS M. RACKEMANN

From the Medical Clinic of the Presbyterian Hospital, Columbia University, New York

The work here reported had to do with the investigation of changes in the cell content of the peritoneum of the guinea-pig (as shown by serial differential counts of the peritoneal fluid made at varying intervals after the intraperitoneal injection of horse serum into guinea-pigs sensitized to horse serum), and of the reaction of the cells to determine whether this reaction differed from that obtained in the normal animal after the injection of horse serum or other non-protein substances.

Studies have been made of the cellular reaction after various injections of foreign protein into the skin. Thus, the von Pirquet reaction to tuberculin in man is known to consist largely of lymphoid cells, while the intradermal introduction of 1 c.c. of horse serum in sensitized rabbits, produces largely a polymorphonuclear reaction, according to Knox, Moss, and Brown.[1] It was thought that by studying the peritoneal fluid of the sensitized guinea-pig, the reaction to intraperitoneal injections might be found in the cells free in this cavity and the progress of the reaction in that case closely followed.

The cellular reaction, both local and general, to injections of various foreign protein substances in animals has been studied by numerous investigators. Most observers, however, have confined their attention to a study of the eosinophilia, either local or general, which may appear under these circumstances.

Schlecht and Schwenker[2] have noticed local collections of eosinophiles in sensitized animals at the site of re-inoculation of foreign protein. They find these in Arthus' phenomenon, in the peribronchial tissue after an intratracheal spray of the homologous protein, and they find them in the "enteritis anaphylactica" of the dog. They have noted, in from 6 to 10 days after intraperitoneal injection of foreign serum in normal animals, a marked local increase of eosinophiles, which is preceded by an increase of the pseudo-eosinophiles. After the second injection of foreign serum, however, the eosinophiles appear much more promptly.

Before studying the changes in the peritoneal fluid after any injection, it is necessary to know the normal relation of one cell type to another in the peritoneal fluid of the guinea-pig. The literature on this is scanty, dealing almost exclusively with the percentage of the eosinophiles.

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[1] Jour. Exper. Med., 1910, 12, p. 562.

[2] Deutsch. Arch. f. klin. Med., 1912, 108, p. 405. Deutsch. Arch. f. inn. Med., 1910, 98, p. 327. Arch. f. exper. Path. u. Pharmakol., 1912, 67, p. 137.

Weinberg and Seguin[3] examined the peritoneal fluid in 150 guinea-pigs. They found eosinophiles in all but 7; 18 of the 150 had over 30% of eosinophiles, and in these animals there was an increase of the eosinophiles of the blood. Stäubli[4] says that normal guinea-pigs have 25-31% of eosinophiles in their peritoneal fluid. Szecsi and Ewald[5] examined the peritoneal exudate of 14 normal guinea-pigs and found the percentage of eosinophiles varying from 3 to 60%.

The peritoneal cavity of normal guinea-pigs always contains sufficient fluid for aspiration with a Wright capillary pipet. This fluid contains normally at least 4 types of cells, namely: polymorphonuclear-neutrophiles, eosinophiles, large mononuclear cells, and small mononuclear cells.

As regards the origin of these cells, the eosinophiles and polymorphonuclears doubtless come from the blood directly, being attracted by eosinotactic substances or by local irritation. Szecsi and Ewald[5] could demonstrate parasites in all cases of peritoneal eosinophilia. But about the mononuclears, large and small, there is some discussion. Before Goldmann's[6] work with vital staining and before the study of animals rendered aleukocytic by means of thorium according to the method of Lippmann and Plesche,[7] there was some doubt about the origin of these two types. Some investigators, notably Weidenreich and Marchand, believed that the large and the small mononuclears were different stages in the development of the same cell, which was originally a local tissue cell. Others, headed by Maximow, thought that since the small mononuclears were morphologically identical with the lymphocytes of the blood, they came from the blood. Others, again, notably Weidenreich, Pappenheim, and Szecsi, divided all these mononuclears into hematogenic lymphatic cells and histiogenic myeloid varieties, and they believed that the epithelium of the serosa was similar in function to the endothelium of the blood vessels and could form new cells and could liberate free "Wanderzellen," which were ordinarily at rest.

Vital stains have demonstrated that both the large and the small mononuclears in the peritoneal exudate are colored, whereas pure lymphocytes, such as are found in the blood, do not take the stain. This seems to prove that both these forms are of histiogenic origin, thus confirming the original unitarian idea of Weidenreich and Marchand.

In the Lippmann-Plesch experiments with animals treated with thorium, the blood of which contained not a single leukocyte, Pappenheim[8] obtained peritoneal exudates filled with lymphoid cells containing carmin. Hence, it is perhaps reasonable to assume that these "small lymphocytes" are not true lymphocytes, but are in fact local autochthonous cells, the so-called histiocytes. Since the large mononuclear cells occur in pericardiac exudates, and since they are different morphologically from any cell, either in the lining membrane of the pericardium or in the blood, it is reasonable to assume at least that they arise neither from the cells lining the serous cavities nor from the cells of the blood itself; their origin can therefore be spoken of simply as "histiogenic."

Throughout the present work the four types of cells have been designated by these titles: (1) polymorphonuclears; (2) eosinophiles; (3) phagocytes (large mononuclears), and (4) lymphocytes (small mononuclears).

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[3] Ann. de l'Inst. Pasteur, 1914, 28, p. 470.
[4] Trichinosis, 1909, p. 214.
[5] Folia haematol., Arch., 1913, 17, p. 167; 1912, 13, p. 1.
[6] Laupp, 1912.
[7] Deutsch. med. Wchnschr., 1913, 39, p. 1395.
[8] Centralbl. f. allg. Path., 1913, 24, p. 997. Folia haematol., Arch., 1913, 17, p. 257.

As a starting point and foundation for further study, differential counts were made of the cells in the peritoneal cavities of 22 normal guinea-pigs of various weights. It is interesting to compare these counts (Table 1). It will be seen that the proportions of different cells varied considerably in different guinea-pigs, and that no fixed normal can be obtained.

TABLE 1

DIFFERENTIAL COUNTS OF THE CELLS IN THE PERITONEAL FLUID OF 22 NORMAL GUINEA-PIGS

Weight in Grams	Percentage of			
	Lymphocytes	Phagocytes	Eosinophiles	Polymorphonuclears
593	44.2	40.4	12.8	2.5
470	12	41	45	2
284	15	57.5	27.5	0
325	23.4	70.2	4.4	2
325	15.5	76	8.5	0
393	52.4	47.6	0	0
684	43.6	56.4	0	0
263	37.4	43	19.6	0
464	6.2	80.8	13	0
515	25.2	63.3	11.5	0
465	7.8	71	21.2	0
323	4.6	79.4	16	0
293	63.6	36.4	0	0
339	24.8	67.6	7.6	0
408	7.6	72	20.4	0
440	7.8	68	24.2	0
280	4	50.2	45	0.8
333	34.8	50.8	14.4	0
357	5.5	73	21.5	0
185	46.1	50.8	3.1	0
235	19.3	73.7	7	0
222	65	33.7	1.3	0
Average	25.71	59.22	14.73	0.34

Polymorphonuclear neutrophiles were absent in all but 4 pigs, and the highest percentage was 2.6. Eosinophiles were absent in 3 pigs. They formed 45% of the cells in 2 pigs, while the next highest count gave 27.5%. The average for the 22 pigs was 14.173% eosinophiles. In only 3 pigs were both polymorphonuclear neutrophiles and eosinophiles absent. The percentage of the small mononuclears and the lymphocytes varied widely, from 65% to 4%. In 4 pigs the proportion was 45% and in 7 it was below 10%. The average for 22 pigs was 25.7% lymphocytes. The proportion of the large mononuclears or phagocytes varied from 80.8% down to 33.7% and averaged 59.2%. If we add the mononuclears, large and small together, we have a much smaller variation, from 100% down to 75%, with only 3 pigs below this. The count in these gave 72.5, 54.2, and 53%, respectively, and the average now becomes 84.9%. Thus most of the cells in the peritoneal cavity of guinea-pigs are of the mononuclear variety.

To demonstrate further these variations in individual guinea-pigs, a normal animal, weighing 684 grams, gave practically the same count as one weighing 185 grams, both with high lymphocytes (43.6% and 46.1%, respectively), while a guinea-pig of 465 grams gave practically the same count as one weighing 323, the proportions of the lymphocytes here being 7.8% and 4.6%. But on the whole, guinea-pigs of the same weight tend to have similar counts.

An interesting observation is that the lower lymphocyte counts tend to occur in the larger guinea-pigs; thus, of 8 pigs weighing over 400 grams, 4 had less than 8% of lymphocytes, while of 14 pigs under 400 grams, only 3 had less than 15% lymphocytes.

No definite influence could be ascribed to the time of year, to the time of day, or to the individual lots of guinea-pigs, and all the guinea-pigs were kept under the same conditions in the laboratory.

Before describing the cellular changes in the peritoneal fluid after the intraperitoneal injection of horse serum into normal and into sensitized guinea-pigs, it is necessary to describe control experiments. Each of these controls was carried out with exactly the same technic in normal guinea-pigs, varying in size but averaging about the same weight as those used later for the horse serum injections. Differential counts, each of at least 500 cells, were made of the peritoneal fluid immediately before the intraperitoneal injection, and at intervals of 15 minutes, 1 hour, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, and in some cases several days afterward. (In some of the earlier controls with non-protein substances these exact time intervals were followed only approximately.)

TECHNIC

The abdomen is carefully shaved, not only to make the introduction of the glass capillary pipette easier, avoiding all hair and extraneous matter, but also to avoid the visible blood vessels, the bleeding from which ordinarily contaminates the fluid. Drops of peritoneal fluid are then mixed on a slide with about equal quantities of an aqueous solution of brilliant cresyl blue in 1:300 dilution and covered with a cover glass. In 5 to 10 minutes all the nuclei are stained and the four main types of cells considered here are readily distinguished. For convenience, these have been here designated as "polymorphonuclears," "eosinophiles," "lymphocytes," and "phagocytes."

Before studying any of the control injections, the peritoneum of a normal guinea-pig, weighing 325 grams, was aspirated repeatedly at the time-intervals enumerated, in order to determine whether repeated punctures alone might cause alterations in the proportion of cells. Such, however, did not occur; differential counts made of samples of these aspirated fluid showed approximately the same proportion of cells in all counts.

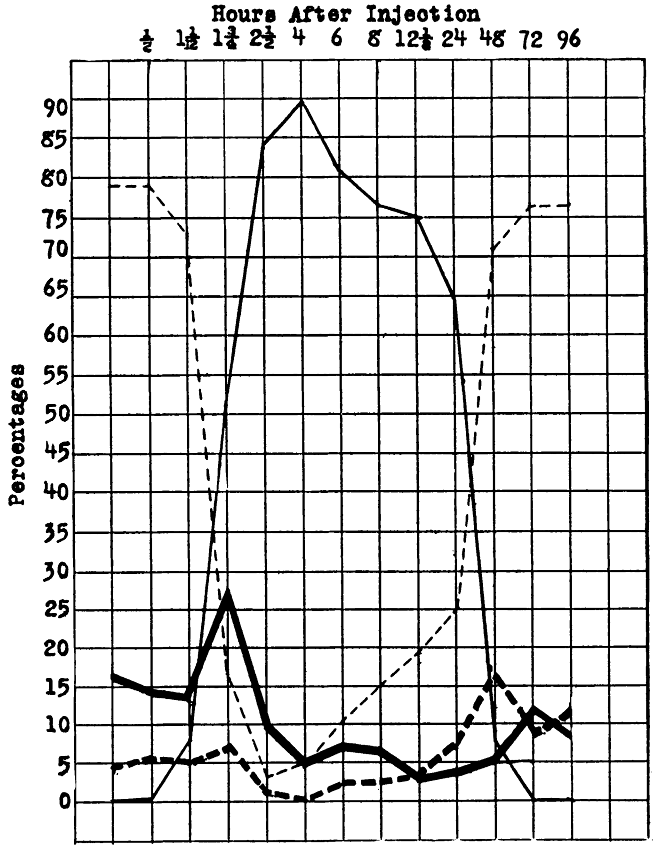

Chart 1.—The changes in the cellular content of the peritoneum of the normal guinea-pig following the intraperitoneal injection of 2 c.c. of 0.85% salt solution. The percentages refer to cells.

KEY TO CHARTS 1, 2, and 3

——— = Polymorphonuclears.
▬▬▬ = Eosinophiles
— - — = Phagocytes.
■ ■ ■ ■ = Lymphocytes.
• • • • • • • = Lymphocytes and phagocytes.

Test substances as controls were injected intraperitoneally into normal guinea-pigs as follows:

(*a*) Two pigs, weighing 325 and 393 grams, respectively, received 1 c.c. salt solution each.

(*b*) Five pigs, varying in weight between 684 and 323 grams, received 2 c.c. salt solution each.

(*c*) One pig of 470 grams received 2 c.c. distilled water.

(*d*) One pig of 344 grams received 1 mg. of histamin suspended in 1 c.c. of salt solution.

(*e*) One pig of 293 grams received 0.1 c.c. of turpentine.

To summarize the results of these preliminary experiments, it may be said that the intraperitoneal injection of these different fluids causes in each case the same general changes in the cell content of the peritoneal fluid of guinea-pigs; the differences in the reactions to each test substance are chiefly of degree; practically all cause a polymorphonuclear rise within 4 hours, and in practically all the reaction is largely over at the end of 24 hours, since at this time the proportions of the different cells tend always to return to the individual normal. Furthermore, after this time daily counts become almost exactly alike and remain the same, even if made perhaps 10 or 20 days after the injections.

As a type of the reaction to these different substances studied, the reaction to the intraperitoneal injection of 2 c.c. salt solution may be described; and to demonstrate the different reactions to the same test substance in different guinea-pigs, Chart 1 is appended, showing the percentage changes after the intraperitoneal injection of 2 c.c. salt solution in a normal guinea-pig.

In the first 15 minutes, the percentage of phagocytes falls perhaps 8 points and that of the eosinophiles (if any are present beforehand), together with that of lymphocytes, rises. This change continues up to 1 hour; and at this time a few polymorphonuclears may be seen, perhaps 6-10%. At the end of 3 hours, there is a striking change; the polymorphonuclear neutrophiles rise from practically nothing to 60 or even 80% with, of course, a corresponding fall in the mononuclear elements, especially marked in the phagocytes. The polymorphonuclears may stay up until the sixth or even ninth hour after injection, or they may fall away again at once.

At the end of 24 hours, the proportions of the cells tend always to return to what they were before injection, and after this time daily counts become almost exactly alike, remaining the same even if made perhaps 10 or 20 days after the injection.

The reaction to the intraperitoneal injection of distilled water is in general much like that to salt solution. Histamin produces similar changes. Turpentine, as may be imagined, causes a high rise in the polymorphonuclears, which reach at the end of 4 hours the proportion of 63%, and remain the predominating cell for from 12 to 24 hours.

It having been determined that the reaction of the cells in the guinea-pig peritoneum was much the same with all these fluids, the following main experiment was carried out:

Ten guinea-pigs sensitized to horse serum were re-injected intraperitoneally with horse serum, the peritoneal fluid being aspirated and studied immediately before the injection and at the following intervals afterward: 15 minutes, 1 hour, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, and in some cases later. Seven of these 10 guinea-pigs were sensitized by an intraperitoneal injection of 0.1 c.c. horse serum, and 3 were sensitized by an intravenous injection of 1 c.c. of horse serum. The sensitizing doses in each of the 10 guinea-pigs were given from 15 to 63 days prior to the second injection. In the first animal 3 c.c. of horse serum were given intraperitoneally at the second injection. This caused well-marked anaphylactic symptoms to appear in 10 minutes; namely, weakness, difficult respiration, and occasional twitching until death occurred 5 hours later. Subsequently a second dose of 2 c.c., given intraperitoneally, invariably produced "severe symptoms" without death. The 3 animals sensitized intravenously had only "slight symptoms."

Each of the 10 experiments was controlled by studying simultaneously or within 24 hours a normal animal of the same weight injected in the same way with the same dose of horse serum. These controls never showed symptoms. In some cases, the total number of cells per cubic millimeter of peritoneal fluid was investigated and from this the actual numbers of each particular cell calculated.

It is interesting to compare these figures with the original percentages, but, in most cases, there is little correspondence between them. This is due to the great variation in the total quantity of the fluid present, so that any great fall in the total number of cells may be real, or it may be apparent because of dilution. Hence, in this work much more stress has been laid upon the percentages than upon the actual numbers.

Preliminary counts immediately before intraperitoneal injections were made both in the sensitized and in the normal guinea-pigs. The counts made of the cells in the peritoneal fluid of the 10 normal controls have been included among the figures given in Table 1 for normal guinea-pigs. When the preliminary counts of the peritoneal fluid of the 10 guinea-pigs sensitized to horse serum (Table 2) are compared with the normal, it is seen that they differ strikingly, in that the fluid averages 10% more eosinophile cells than the normal (Table 3). This has been observed by other workers and has been commented on especially by Weinberg and Seguin.

TABLE 2

DIFFERENTIAL COUNTS OF CELLS IN THE PERITONEUMS OF GUINEA-PIGS SENSITIZED TO HORSE SERUM

Weight in Grams	Percentage of			
	Lymphocytes	Phagocytes	Eosinophiles	Polymorphonuclears
...	13.2	47.5	38.5	0.8
300	20	40.6	39.4	0
342	28.2	46.4	25.4	0
460	57.7	39.5	2.8	0
292	5.8	74.0	20.2	0
324	4.2	70.6	25.2	0
375	54	34.8	11.2	0
214	5.2	58.4	36.4	0
209	5.2	55.8	39.0	0
237	6	82.3	11.2	0.5
Average	19.95	54.99	24.93	0.13

TABLE 3

COMPARISON OF THE AVERAGE PERCENTAGES OF CELLS IN THE PERITONEAL CAVITY OF NORMAL GUINEA-PIGS WITH THAT OF GUINEA-PIGS SENSITIZED TO HORSE SERUM

	Average Percentage of Cells in Peritoneal Cavity of	
	22 Normal Guinea-Pigs	10 Guinea-Pigs Sensitized to Horse Serum
Lymphocytes	25.71	19.95
Phagocytes	59.22	54.99
Eosinophiles	14.73	24.93
Polymorphonuclears	0.34	0.13

The intraperitoneal injection of 2 c.c. of horse serum into sensitized guinea-pigs causes a somewhat different cellular reaction from that observed after the intraperitoneal injection of the same amount of horse serum into normal guinea-pigs. The effect of the intraperitoneal injection of horse serum into normal guinea-pigs does not differ from that obtained with the other control fluids, such as salt solution, distilled water, and dilute turpentine.

Charts 2 and 3 demonstrate the changes observed in the cell content of the peritoneal fluid after injections of horse serum, both in the normal and in the sensitized guinea-pig. In both the sensitized and the normal guinea-pig, within 15 minutes after the injection of horse serum, there is a marked drop in the percentage of phagocytes with a marked rise in that of lymphocytes, while that of eosinophiles remains about the same, altho tending to rise in the sensitized animal. The fluid is increased in amount in both and has comparatively very few cells, the total number of cells decreasing in a striking manner—this decrease being perhaps more pronounced in the sensitized guinea-pig. At the end of an hour, the abdomen in the sensitized animal is almost tense with fluid and there are still very few cells. The percentage of lymphocytes now diminishes while that of the phagocytes and of the eosinophiles increases. In the normal animal this increase in the percentage of eosinophiles is more rapid than in the sensitized one.

In 3 hours after the injection of horse serum, the peritoneal fluid of the normal guinea-pig contains great numbers of polymorphonuclear cells, so that they form 50 to 80% of the total number; whereas in the sensitized animal at this time few of these cells are to be found and the mononuclears still predominate. In the chart shown, the polymorphonuclears formed but 9% of the cells in the peritoneum of the sensitized guinea-pig, while the mononuclears made up 53%. At the end of 6 hours the polymorphonuclears usually reached their highest percentage in the normal guinea-pig; whereas in the sensitized animal, tho they are increasing, they still form less than half the total cells. In 9 hours, the percentage of polymorphonuclears in the normal guinea-

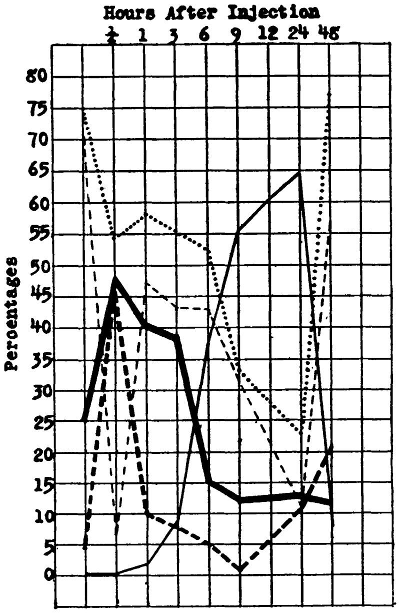

Chart 2.—The variations in the proportions of cells in the peritoneal fluid of the normal guinea-pig after an injection of 2 c.c. of normal horse serum.

pig may begin to decline, but in the sensitized animal it may still be slowly rising, tho even at this late date the rise is scarcely comparable to that observed in the normal guinea-pig at the end of 3 hours. Differential counts at later dates show that both in the normal and in the sensitized guinea-pig the proportions of the cells tend to return to their individual normals after from 24 to 48 hours, the polymorphonuclears disappearing, the mononuclears reappearing, tho this may be somewhat slower in the sensitized than in the normal guinea-pig. After this time no definite changes can be observed in the proportions of the cells of the peritoneal fluid of either group of animals.

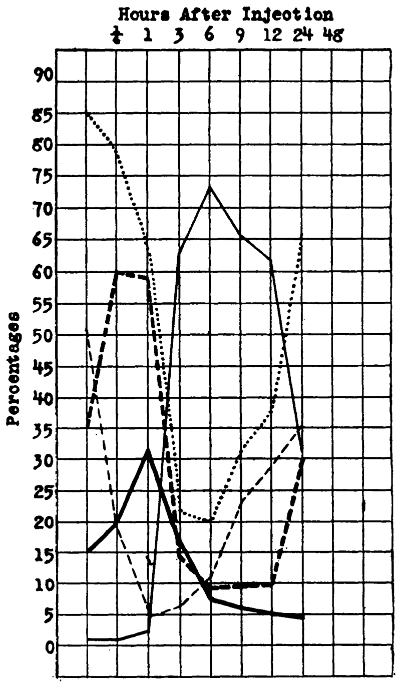

Chart 3.—Variations in the proportions of cells in the peritoneal fluid of the sensitized guinea-pig after an injection of 2 c.c. of normal horse serum.

The changes in the total numbers of cells in the peritoneal fluid, as determined by counts made with the Thoma-Zeiss hemocytometer, have thrown no further light on the subject. In both sensitized and normal animals the total number of cells falls rapidly in 15 minutes. In one normal guinea-pig the number fell from about 11,500 to 700; in a sensitized guinea-pig, from about 27,500 to 12,000. During the period at which the percentage of polymorphonuclears increases the total cell count increases. There is a second fall at about 9 to 12 hours during the period in which the percentage of polymorphonuclears decreases, with a final rise as the cell proportions return after from 24 to 48 hours to the individual normal.

It is evident from this description and the charts that the reaction of the peritoneum of the normal guinea-pig towards horse serum, is not essentially different from that observed towards salt solution, water, or dilute turpentine. The peritoneum of the sensitized guinea-pig, on the other hand, reacts very differently from the normal to the second injection of horse serum. Tho there are certain minor and inconstant differences, the striking and perhaps important difference following the intraperitoneal injection of horse serum is the delay in the appearance of polymorphonuclear leukocytes in the peritoneal fluid of sensitized guinea-pigs. In the normal animal these cells are present in overwhelming proportion within 3 hours after the intraperitoneal injection of horse serum, whereas in the sensitized guinea-pig they may scarcely make their appearance before this time and rarely occur in great excess before 6 to 9, or even 12, hours.

The delay in the polymorphonuclear response of the sensitized animal is of interest in the light of the experiments reported by Zinsser and Dwyer.[9] They found that a proteotoxin, made by mixing suspensions of typhoid bacilli and normal guinea-pig serum, has the power to increase markedly the virulence of other bacteria. They found that this aggressive action of the proteotoxin is not due to the inhibition of phagocytosis; as the animals injected with it show a leukopenia, they explain the action as due to a poisoning of those tissues which ordinarily react with mobilization of the leukocytes from the circulation.

It is highly probable that a close analogy exists between the reaction of the peritoneum of the guinea-pig towards the proteotoxin of Zinsser and Dwyer[9] and the anaphylactik shock following the intraperitoneal injection of horse serum, and it is not unlikely that any condition which interferes with the rapid outpouring of leukocytes into the peritoneal cavity will assist in the spread of infection or growth of bacteria introduced into the peritoneal cavity at this time. The reaction, therefore, of the peritoneal cavity of the sensitized guinea-pig to injections of horse serum, is very different from the reaction of the skin under the same conditions. This may be partly due to the rapid

[9] Jour. Exper. Med., 1914, 20, p. 582.

absorption from the peritoneal cavity of any toxic substances that are formed. With the intracutaneous injection the absorption would be much slower.

CONCLUSIONS

There is no fixed, normal percentage relationship between the types of cells in the peritoneal fluid of normal guinea-pigs.

Old guinea-pigs tend to show a smaller proportion of small mononuclear cells than do young animals.

The peritoneal fluid of guinea-pigs sensitized to horse serum contains a higher percentage of eosinophiles than is found in the normal animal.

The reaction of the peritoneum in normal guinea-pigs to normal salt solution, water, histamin, turpentine, and horse serum, is qualitatively much the same in each case, and consists in the rapid disappearance of the mononuclear cells and the accumulation within 3 hours of polymorphonuclear leukocytes in overwhelming proportions.

The peritoneum of the guinea-pig sensitized to horse serum reacts to an injection of horse serum differently from the normal, inasmuch as the appearance of the polymorphonuclear leukocytes is much delayed.